

JAN-EDVIN OLSSON

**STUDIES ON BETA-TRACE PROTEIN.
DISTRIBUTION, SYNTHESIS AND META-
BOLISM IN NORMAL AND PATHOLOGICAL
CONDITIONS**

LUND 1973

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The present thesis is based on the following papers:

- I. H. Link and J-E. Olsson:
Beta-trace protein concentration in CSF in neurological disorders.
Acta Neurologica Scandinavica, 48, 57-68, 1972.
- II. J-E. Olsson and L. Nord:
Immunochemical and immunofluorescence studies of beta-trace protein in different species and organs, with special reference to the central nervous system.
Journal of Neurochemistry, In press.
- III. J-E. Olsson and H. Link:
Distribution of serum proteins and beta-trace protein within the nervous system.
Journal of Neurochemistry, 20, 837-846, 1973.
- IV. J-E. Olsson, C. Blomstrand and K.G. Haglid:
The cellular distribution of beta-trace protein in CNS and brain tumours.
Journal of Neurology, Neurosurgery and Psychiatry, In press.
- V. J-E. Olsson and M. Sandberg:
Demonstration of synthesis of beta-trace protein in different tissues of squirrel monkey.
Scandinavian Journal of Immunology, In press.
- VI. J-E. Olsson, H. Link and B. Nosslin:
Metabolic studies on [¹²⁵I] beta-trace protein, with special reference to synthesis within the central nervous system.
Journal of Neurochemistry, In press.

In the text these papers will be referred to by these numbers.

INTRODUCTION

The total protein concentration in normal human cerebrospinal fluid (CSF) is about 1/200 of that in serum. Electrophoretic investigations have revealed a protein pattern of CSF, similar, but not identical to that of serum. The blood-brain barrier seems to be more easily passable for proteins with a low molecular weight. Therefore, certain high molecular proteins, e.g. immunoglobulin M with a molecular weight of about one million can not be demonstrated in normal CSF and immunoglobulin G with a molecular weight of about 150,000 occurs in normal CSF in a lower concentration than would be anticipated on the basis of the concentration in serum, whereas albumin, with a molecular weight of 69,000 is relatively more represented in CSF than immunoglobulin G.

The introduction of immunoelectrophoresis should enable to answer the question of the eventual occurrence in CSF of proteins specific to the central nervous system (CNS). With the use of antisera against human CSF, more than ten such proteins have been claimed to occur (Laterre, 1965). With this technique and a rabbit antiserum against human CSF, two precipitation lines in the beta- and the gammaglobulin regions could be found in concentrated CSF, but not in serum (Clausen, 1961). One of these lines reached from the α_2 -region to the middle of the gammaglobulin region, and the precipitation line was nearest to the antibody trough in the betaglobulin and the anodal part of the gammaglobulin region. Fig. 1 shows this precipitate and it was suggested that the protein, which gave this precipitate should be called 'beta-trace protein' (Hochwald and Thorbecke, 1962). The discovery in human CSF of beta-trace protein was later confirmed in other investigations (Laterre and Heremans, 1963; Link, 1965; Frick, 1965). At first, this protein was thought to be specific for CSF, but it was soon detected in urine (Hochwald

and Thorbecke, 1962; Laterre, Heremans and Carbonara, 1964) and in ascitic fluid from two patients (Hochwald and Thorbecke, 1962). A precipitation line, almost identical with that of beta-trace protein, had earlier been detected in human perilymphatic fluid with an antiserum against human CSF (Chevance, Galli, Jeanmarie and Gerard, 1960) although these authors could not really evaluate this finding. With the immunoelectrophoretic techniques, the protein could not be found in concentrated serum, but after pooling, concentration, and gel filtration, beta-trace protein could be demonstrated in a fraction of normal serum (Walravens, Laterre and Heremans, 1968).

The mean concentration of beta-trace protein in CSF was determined in two materials consisting of 22 and 12 healthy adult persons, respectively, at 2.6 and 4.0 mg per 100 ml (Link, 1967; Pepe and Hochwald, 1967). Although two different techniques were used, the results indicated that beta-trace protein is one of the major CSF proteins, constituting about 7 - 10 per cent of the total protein concentration in CSF. Beta-trace protein could be quantified in unconcentrated serum from patients with impaired renal function, but not in normal serum, where the concentration was less than 0.5 mg per 100 ml (Ericsson, Link and Zettervall, 1969). The protein was demonstrated in about hundredfold concentrated urine from 16 out of 17 healthy persons and the amount excreted per 24 hours was found to be at most 9.5 mg (Ericsson et al., 1969).

Beta-trace protein was also found in extracts of water-soluble proteins of human brain (Laterre et al., 1964, Penny and Osserman, 1971), where the concentration varied between 0.10 and 0.22 per cent of the total water-soluble protein concentration (Link, 1972). These findings, together with the high concentration in CSF, gave rise to the suspicion that the protein was synthesized within the CNS and then conveyed to CSF. However, by measuring the incorporation of radioactive amino acids into tissue cultures of rhesus monkeys, no sure synthesis of beta-trace protein could be found in 12 cultures of brain and in four out of five cultures of the choroid plexus, whereas one of these cultures

showed signs of synthesis (Hochwald, Pepe and Thorbecke, 1967). A distribution of beta-trace protein outside the CNS was suggested by Penny and Osserman (1971), who used the Ouchterlony immunodiffusion technique and found weak precipitates between extracts of kidney, fimbriae of the Fallopian tubes, ovary, testis, pancreas, and leucocytes, and an antiserum against beta-globulins from the urine of a patient with multiple plasmocytoma. This antiserum showed a certain immunological identity with a specific antiserum against beta-trace protein received from Hochwald. It is notable that two precipitation lines were found between the antiserum and extracts of testis (Penny and Osserman, 1971). Contrary to this investigation, Laterre et al., (1964) using immunoelectrophoresis and an antiserum against urine found no beta-trace protein in extracts of kidney. However, these findings must be somewhat doubtful, as the antisera were prepared from animals, immunized with urine, where the concentration of beta-trace protein is low and where a lot of low molecular weight proteins exist. These proteins may give immunological cross-reactions with beta-trace protein and this can explain the double precipitates found against the extract of testis. A synthesis of beta-trace protein in the genital organs has been proposed by Hochwald et al., (1967) who found signs of synthesis in one examined culture of each testis and spermatocord and two cultures of the fimbriae ovary of the Fallopian tube of four examined. Eight cultures of the ovaries showed no signs of synthesis of beta-trace protein. Nor did cultures of liver and kidney (Hochwald et al., 1967).

The high concentration of beta-trace protein in CSF made it possible to isolate the protein from pooled, concentrated human CSF by means of ion chromatography and stepwise elution (Hochwald and Thorbecke, 1963, a). This fraction was not quite pure and gave two peaks by ultracentrifugation. By adding gel filtration to the isolation procedure, a pure fraction was obtained which gave only one peak of 2.3 S at ultracentrifugation (Link, 1965, 1967). The molecular weight of the protein was calculated

to be 31,000 (Link, 1967). The amino acid composition was determined by Link (1967) and the amino acids occurring most frequently were found to be threonine, serine, glutamic acid, alanine, and leucine. When the purified protein is analysed with electrophoresis on different supporting media, usually several bands are seen. On agar gel electrophoresis, beta-trace protein migrates as three evenly distributed distinct bands between the cathodal transferrin band, named the 'tau'-fraction and the band corresponding to immunoglobulin G (γ_4 -fraction) (Link, 1967) (Fig. 1). Three bands are also seen on starch gel electrophoresis (Link, 1967), on polyacrylamide gel electrophoresis (Blomstrand and Olsson, unpublished), whereas a diffuse smear is seen in the gammaglobulin region on cellulose acetate electrophoresis (Link, 1967). On agarose gel electrophoresis, beta-trace protein remains around the application place, without separation into visible bands (Link, 1973). If beta-trace protein is treated with neuraminidase, the immunoelectrophoretic precipitation line moves towards the cathode (Hochwald and Thorbecke, 1963,b) and two of the three bands seen on the agar gel electrophoresis disappears, whereas the third and most cathodal fraction becomes stronger (Link, 1967). These findings indicate that beta-trace protein is a glycoprotein and contains sialic acid. A rather high content of about 7.96 g glucosamine per 100 g protein has also been found (Link, 1967).

The function of beta-trace protein is unknown. The protein has been compared with the immunoglobulins, because of its electrophoretic mobility in the gammaglobulin region and of its low molecular weight, which is close to that of light chains of immunoglobulins and the β_2 -microglobulin (Berggård and Bearn, 1968), which was recently found to have an amino acid sequence similar to the light chains (Peterson, Cunningham, Berggård and Edelman, 1972). However, no certain common properties have been found between on one hand, beta-trace protein and on the other, heavy and light chains of the immunoglobulins (Link, 1967) and β_2 -microglobulin (Walravens, Laterre, Estas and Heremans, 1967).

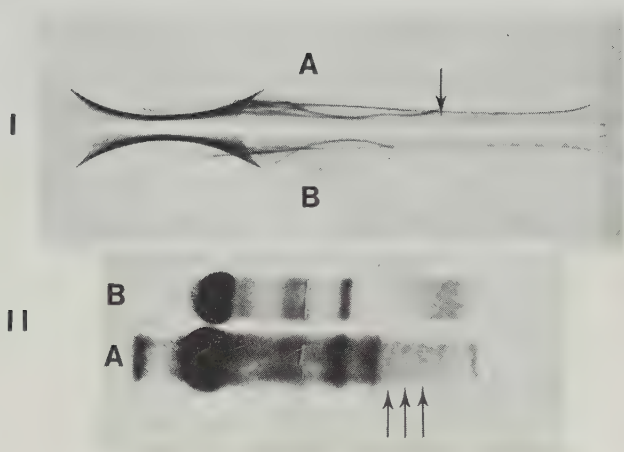


Fig. 1. Immuno-electrophoresis (I) and agar gel electrophoresis (II) of normal human cerebrospinal fluid (A) and serum (B). The trough in the middle of the immuno-electrophoresis contains anti-serum against cerebrospinal fluid. Arrows denote beta-trace protein.

There are few investigations of beta-trace protein in pathological conditions. Increased concentrations in CSF were found in patients with cerebral vascular diseases and in patients with renal failure (Ericsson et al., 1969). Slightly, but not significantly, higher concentrations in CSF were found in patients with cerebral tumours (Pepe and Hochwald, 1967; Ericsson et al., 1969) and with multiple sclerosis (Link, 1967) and in infants with hydrocephalus (Pepe and Hochwald, 1967). Beta-trace protein was quantified in serum from patients with renal diseases; and serum concentrations, even higher than in normal CSF, were found in bilaterally nephrectomized patients and in patients with serum creatinine values higher than 10 mg per 100 ml (Ericsson et al., 1969). Urine from patients with renal failure was found to contain more beta-trace protein than normal urine (Walravens et al., 1967; Ericsson et al., 1969), as well as

urine from patients with cerebral vascular disease and cerebral tumour (Ericsson et al., 1969).

All these data show that beta-trace protein is present in a rather high and constant concentration in human CSF, whereas the concentration in human serum is considerably lower, and it was only possible to determine the concentration of beta-trace protein in serum in patients with impaired renal function. The normal urinary excretion is less than 9.5 mg per 24 hours, but it increases when there is a tubular damage. Beta-trace protein has been found in extracts of water-soluble proteins of human brain and probably also in extracts of urogenital organs. In some patients with destruction of brain tissue, the protein is probably delivered to CSF and elevated concentrations of beta-trace protein can be found in the CSF. Beta-trace protein can be isolated from human CSF and is relatively well characterized.

The purpose of the investigations included in this thesis was to study the concentration of beta-trace protein in CSF in different ages and in different pathological conditions; to establish the presence and the concentration in normal human serum; to determine the distribution in different human organs and especially in different parts of the CNS; to study the synthesis and the metabolism of protein, and to investigate the possible presence of beta-trace protein in other species.

MATERIALS

Human CSF and serum were obtained from patients at the Department of Neurology, University Hospital, Lund. Normal human tissues were received at autopsies at the Department of Pathology, University Hospital, Lund. Brain tumours were obtained at operations at the Department of Neurosurgery, Sahlgren's Hospital, Gothenburg. Normal tissues were also received from squirrel and rhesus monkeys, calves, rabbits, and mice.

Beta-trace protein was isolated from pooled, concentrated human CSF by ion exchange chromatography and gel filtration according to Link (1965, 1967).

A specific rabbit antiserum against beta-trace protein was prepared as described by Link (1967) or in the separate papers. Antisera against other proteins were prepared as described in the separate papers.

METHODS

CSF was obtained by lumbar puncture, performed according to Antoni (1923) and with the modification by Höök (1957).

Extracts of water-soluble proteins were prepared by homogenization of tissues at 4°C with 0.25 M-sucrose (Link, 1972).

Preparations of fractions enriched in neuronal and glial cells were performed with gradient ultracentrifugation (Blomstrand and Hamberger, 1969).

Determination of beta-trace protein was performed:

a. Qualitatively with double diffusion in agar gel (Ouchterlony, 1948).

b. Quantitatively with single radial immunodiffusion on agar according to Mancini, Vaerman, Carbonara and Heremans (1964) with the modification by Link (1967). The reliability of the quantitation of beta-trace protein in CSF was tested by Link (1967). In order to test the reliability of the serum quantitations, known amounts of beta-trace protein were added to samples of normal human serum. Quantitation of beta-trace protein in these samples was then carried out, and the obtained results were compared with those from the simultaneous quantitations of beta-trace protein in the same samples of serum, but free from additional beta-trace protein. The mean of the calculated values,

did not differ by more than 22 per cent from the expected values.

c. Quantitatively by complement fixation, as described by Moore and Perez (1966) and modified by Haglid, Stavrou, Rönnbäck, Carlsson and Weidenbach (1973), for determination of beta-trace protein in extracts of CNS cellular fractions and brain tumours.

Total protein concentration was determined by the method of Lowry, Rosebrough, Farr and Randall (1951), either modified as described by Lous, Plum and Schou (1956) or by Legget Bailey (1962).

Immunofluorescence investigations were performed with the sandwich (two-layer) method on cryostat sections, mainly according to Conns (1957, 1958) but with some modifications described in papers No. II and IV.

Labelling of beta-trace protein with ^{125}I was performed with the iodine monochloride method of McFarlane (1958). Paper VI describes the turnover studies carried out with the ^{125}I -labelled protein.

Synthesis of beta-trace protein was investigated by culturing different monkey tissues in Eagle's medium and measuring the incorporation of ^{14}C -labelled amino acids. Paper No. V describes the method.

The statistical calculations were performed with Student's t-test or the non-parametric, Wilcoxon's rank sum test.

DISTRIBUTION OF BETA-TRACE PROTEIN

a. Cerebrospinal fluid:

In a material consisting of 59 'healthy' adult persons, the concentration of beta-trace protein in CSF varied between 1.4 and 4.0 mg per 100 ml, with a mean value of 2.6 mg per 100 ml,

a standard deviation of 0.6 mg per 100 ml, and a standard error of mean of ± 0.08 mg per 100 ml (paper I). As Figure 2 shows, the values were fairly constant up to the age of 50 years, but increased thereafter. The concentration of beta-trace protein in CSF over 50 years of age differed significantly ($p < 0.001$) from that below 50 years of age.

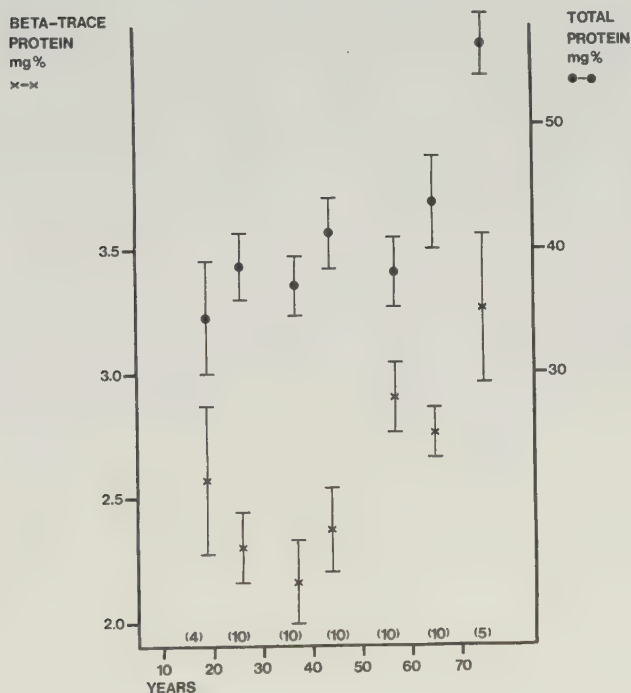


Figure 2. The mean concentrations $\pm$ S.E.M. of beta-trace protein and total CSF protein in different age groups. Figures in parentheses give the number of cases within each group. Each marking refers to the mean age of each group.

b. Serum:

The concentration of beta-trace protein in serum was determined in 25 'healthy' persons and varied between 0.26 and 0.60 mg per 100 ml, with a mean value of 0.39 mg per 100 ml and a standard error of mean of $\pm$ 0.016 mg per 100 ml (paper VI). No variation with age was seen.

c. The nervous system:

The distribution of beta-trace protein was qualitatively investigated in human and monkey brains by immunofluorescence (paper II). Sections through cerebrum, cerebellum, basal ganglia, and spinal cord showed specific immunofluorescence for beta-trace protein, whereas sections through choroid plexus and femoral nerve did not show any specific immunofluorescence. Sections through white CNS matter showed the brightest fluorescence, but also in sections of grey matter, some fluorescence occurred, probably in the fibre tracts.

Beta-trace protein was quantitatively determined in extracts of water-soluble proteins from different parts of the nervous system (paper III). Significantly higher concentrations were found in white CNS matter than in grey, the concentration of beta-trace protein in white CNS matter being about twice as much as that in grey. The concentration in basal ganglia was lower than in white CNS matter, but significantly higher than in grey. The highest values were found in pooled materials of the choroid plexus and the lowest values in pooled materials of the femoral nerve. Four foetal brains from the 26th to the 35th week were investigated. In these brains, the concentration of beta-trace protein increased with age ($r=0.81$).

The cellular distribution of beta-trace protein within the CNS was studied in paper IV. Fractions enriched in glial cells contained about three times as much beta-trace protein as fractions enriched in neuronal cells. This applied to both man and monkey. In sections of cerebral matter of squirrel monkeys, specific immunofluorescence for beta-trace protein was seen in cells of

a morphological appearance, such as that of astrocytes and oligodendrocytes; in sections of cerebellar matter, no immunofluorescence could be seen in the Purkinje cells, whereas specific immunofluorescence was seen in the granular layer. Beta-trace protein was also quantitatively determined in extracts of water-soluble proteins from various human brain tumours. Such of glial cell origin contained considerably higher amounts of beta-trace protein, when compared with other brain tumours. In gliomas, the concentration of beta-trace protein in extracts of water-soluble proteins was 58 times as high as that in extracts of other brain tumours.

d. Tissues outside the nervous system:

Extracts of water-soluble proteins from different human tissues, such as liver, kidney, skeletal muscle, pancreas, testis, epididymis, spermatic cord, ovary, and fimbriae ovary of the Fallopian tube were tested by the Ouchterlony double diffusion technique in agar, to study whether they contained beta-trace protein. Precipitation lines were found between antiserum against beta-trace protein and extracts of the genital organs (paper II). An immunological identity was found between these precipitates and the precipitation line found between CNS tissue and the antiserum against beta-trace protein (Figure 3).

Qualitative immunofluorescence studies on these tissues showed specific immunofluorescence for beta-trace protein in the stroma of epididymis, whereas sections through the other tissues did not show any certain specific fluorescence (paper II). Quantitative investigations of beta-trace protein by means of single radial immunodiffusion in agar in the extracts of these tissues showed concentrations of the protein in extracts of epididymis almost as high as in CNS white matter (paper II). Other genital tissues and liver contained rather high absolute concentrations of beta-trace protein, whereas the concentration in relation to the total water-soluble protein concentration was low. No detectable amounts of beta-trace protein were found in extracts of skeletal muscle and pancreas.

The high content of beta-trace protein in genital organs is reflected in seminal fluid. In 19 adult men with normal number and appearance of sperm cells, the concentration of beta-trace protein varied between 1.0 and 17.5 mg per 100 ml, with a mean of 6.6 mg per 100 ml and a standard error of mean of ± 1.137 mg per 100 ml (Olsson, unpublished).

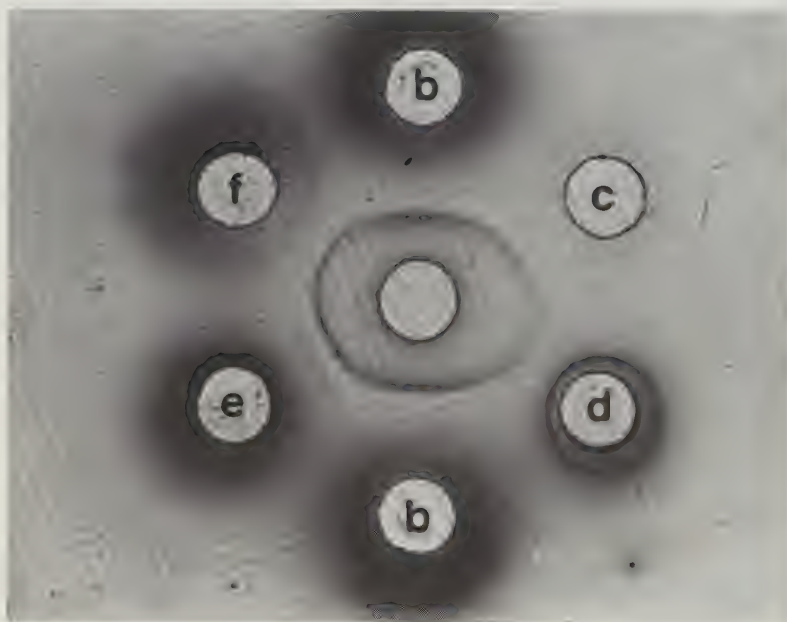


Figure 3. Immunodiffusion plate illustrating the immunological reactions between extracts of different human organs. Peripheral wells contain: (b) human white cerebral matter; (c) femoral nerve; (d) ovary; (e) testis; (f) spermatic cord. All the preparations were concentrated to 4 g protein per 100 ml. Central well contain undiluted antiserum against human beta-trace protein.

e. Different species:

Extracts of water-soluble proteins of brains from squirrel and rhesus monkeys, calves, rabbits, and mice were tested with an antiserum against human beta-trace protein by the Ouchterlony double diffusion technique in agar (paper II). Immunological identity was found only between the monkey brains and the human CSF and CNS.

Discussion:

Human beta-trace protein shows an immunological identity with monkey beta-trace protein, but not with a possible presence of a similar protein in other investigated species. This finding restricts experimental studies to monkeys.

Beta-trace protein has been found in several body fluids. The highest concentration in relation to the total protein content in the fluid is found in CSF, where beta-trace protein constitutes about seven per cent of the total protein concentration. In seminal fluid, beta-trace protein amounts to about one per mille of the total protein content, whereas the concentration of beta-trace protein in serum is only about 1/20,000 of the total protein content.

Beta-trace protein seems to exist in several tissues of the human body, although the largest amounts are found in the CNS and in the genital organs. Within the CNS, the localization seems to be mostly within the white matter, predominantly in the glial cells, such as astrocytes and oligodendrocytes, although the protein to some extent is also found in neuronal fractions. The distribution of beta-trace protein within the CNS is rather similar to that of the brain-specific 'S-100' protein (Moore, 1965), which is slightly smaller than beta-trace protein and has a molecular weight of 19,500 - 24,000 (Stewart, 1972; Moore, 1969). Several studies have described a glial cell origin of S-100 protein; it is also present in glial cell tumours of human brain (Hydén and McEwan, 1966; Benda, 1968; Haglid and Carlsson, 1971; Haglid et al., 1973), although

it is also claimed to be present to some extent in neurons (Hydén and McEwen, 1966; Packman, Blomstrand and Hamberger, 1971; Haglid and Stavrou, 1973). The S-100 protein is very sparsely represented in CSF and seems to be rather fixed to the CNS cells, whereas beta-trace protein seems to be more loosely bound to the cells and leaks out to the CSF. The finding of large amounts of beta-trace protein in the genital organs, especially the epididymis, shows that the protein is not brain specific. The high concentrations of beta-trace protein in CSF and CNS, however, make the protein as interesting as the brain specific proteins, and the occurrence of high amounts of beta-trace protein in the genital organs might facilitate investigations of the function of the protein.

SYNTHESIS AND METABOLISM OF BETA-TRACE PROTEIN

a. Metabolism in serum and CSF:

The intravascular metabolism of beta-trace protein was studied in three patients after intravenous injection of ^{125}I -labelled beta-trace protein (paper VI). In one of the patients, a cerebral embolism caused a moderate hemiparesis ten days before the investigation, whereas the other two patients suffered from a benign form of multiple sclerosis and were almost without symptoms at the time of the investigation. All the patients had a normal total CSF protein content and a normal serum creatinine. As Fig. 4 shows, the protein-bound radioactivity in serum decreased rapidly. The injected ^{125}I -labelled beta-trace protein was excreted in urine, mostly as free iodide. Only about 5 per cent of the total radioactivity excreted in urine per 24 hours was as protein-bound radioactivity. The excretion rate was rapid: already after 24 hours, about 80 per cent of the injected dose was recovered in urine. When the study was terminated, after 96 hours, almost all of the injected radioactivity had been excreted in urine (Fig. 5).

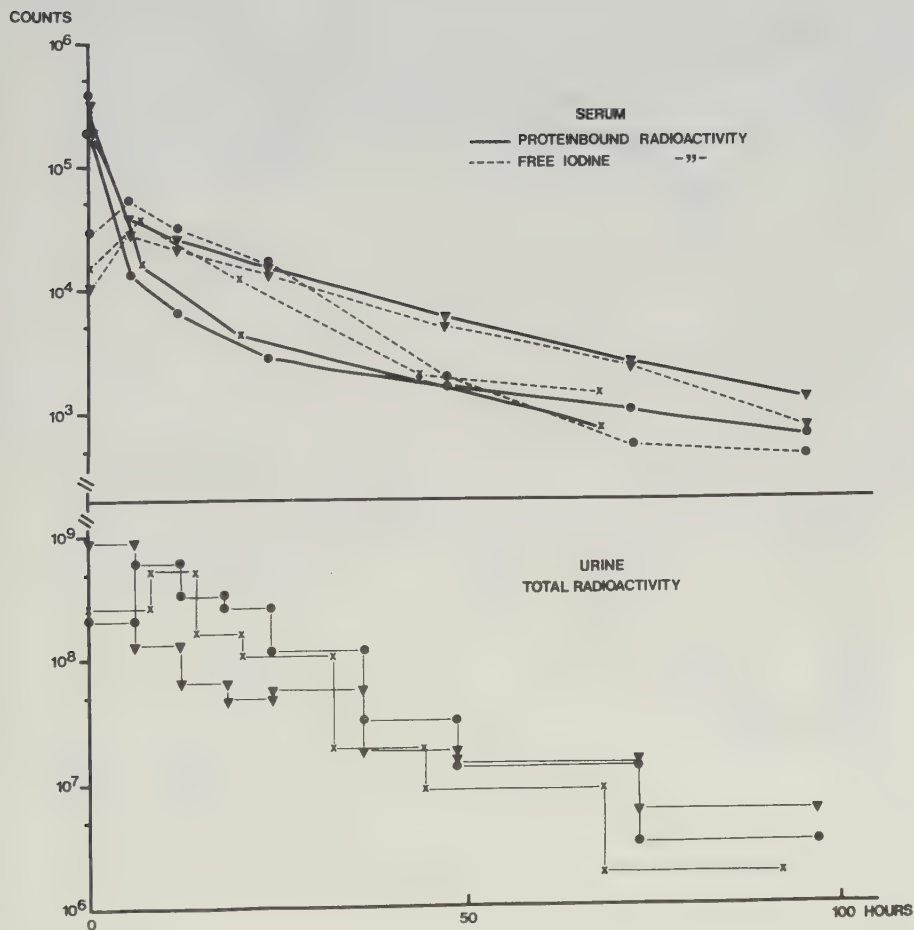


Fig. 4. Curves from the study after intravenous injection of ^{125}I -labelled beta-trace protein, in patients No. 1 (X-X); No. 2 (●-●); and No. 3 (▼-▼). The upper part of the figure contains curves of protein-bound and free iodine radioactivity (counts per 3 ml and 10 min.) in serum, whereas the lower part of the figure contains the urinary excretion of radioactivity (counts per 6 hours).

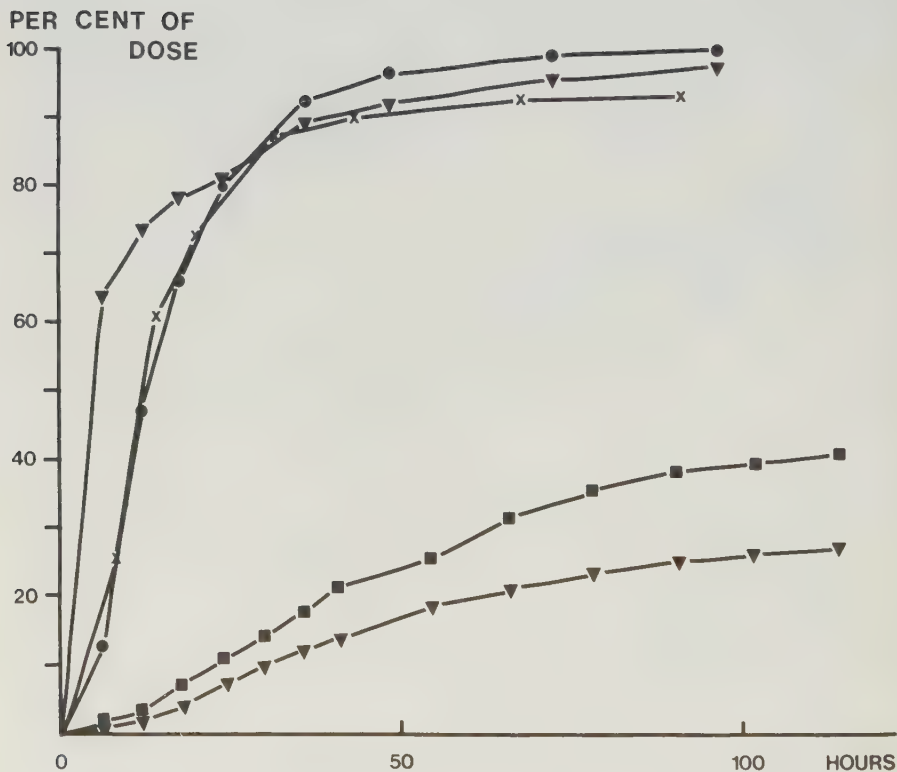


Fig. 5. Curves of cumulative urinary radioactivity excretion in patients No. 1 (X-X); No. 2 (●-●); No. 3 (▼-▼); and No. 4 (■-■). The three upper curves show the urinary excretion after intravenous injection of ^{125}I -labelled beta-trace protein, whereas the two lower curves show the urinary excretion after injection into the lumbar spinal space.

The intravascular turnover time was calculated from the area under the curve of the protein-bound radioactivity in serum according to Nosslin (1964). The turnover rate was rapid and did not significantly differ between the three patients. A mean turnover time in serum of 1.2 hours was calculated. With a mean concentration of beta-trace protein in serum of 0.4 mg per 100 ml and a mean serum volume of 3,000 ml, about 240 mg beta-trace protein is metabolized per 24 hours.

^{125}I -labelled beta-trace protein was also injected into the lumbar spinal space in two patients who had cerebral infarctions. Both patients had a normal total CSF protein concentration and a normal serum creatinine. One of them also participated in the intravenous study. Fig. 6 shows the fate of the labelled protein. The protein-bound radioactivity decreased rather rapidly in CSF, appeared in serum, and was excreted in urine. The urinary excretion rate, however, was slower than after intravenous injection, and about 40 per cent of the injected dose was recovered in urine when the study was terminated 114 hours after the intraspinal injection (Fig. 5).

Calculation by the same method as for the intravascular turnover time determined the intraspinal turnover time at about three hours.

b. Synthesis in man and monkey:

The rapid turnover times of beta-trace protein in both human serum and CSF compared with the considerable differences in concentration of beta-trace protein between CSF and serum constitute indirect proofs of a synthesis of the protein within the CNS.

Direct proofs of synthesis of beta-trace protein within the CNS were obtained by culturing different tissues of squirrel monkeys for 24 hours in Eagle's minimum essential medium together with ^{14}C -labelled valine, threonine, and leucine, amino acids chosen among the most common in beta-trace protein. The synthesized beta-trace protein in the supernatants of the cultures

was precipitated with a rabbit antiserum against beta-trace protein, and the incorporated radioactivity was measured. Normal rabbit serum and antiserum against beta-trace protein, absorbed with pure beta-trace protein, were used as controls. (paper V). Synthesis of beta-trace protein was found in supernatants of white CNS matter, whereas a probable synthesis was found in supernatants of spinal cord, basal ganglia, liver, testis, epididymis, spermatic cord, and fimbriae ovary of the Fallopian tube. Supernatants of cultures of grey CNS matter, kidney, skeletal muscle, and peripheral nerve did not show any sign of synthesis of beta-trace protein. As the cellular density was different in the studied cultures and the total synthesized protein differed in the cultures, no certain quantitative conclusions could be drawn from this investigation of monkey tissues. However, as the turnover time of beta-trace protein in human CSF was found to be about three hours, the mean concentration of beta-trace protein in CSF is 2.6 mg per 100 ml, and the CSF volume is about 150 ml, about 30 mg beta-trace protein being metabolized within the CSF per 24 hours. This amount is probably synthesized within the CNS; thus about one eighth of the daily synthesized beta-trace protein in the body is derived from the CNS.

With the same technique, suspensions containing about 10^7 human lymphocytes were cultured for 24 hours. The lymphocytes were received from three healthy persons and from three patients with multiple sclerosis. In four of the cultures, there was a synthesis of beta-trace protein (the specific precipitated radioactivity exceeded the nonspecific by more than double); in one of the cultures, there was a probable synthesis (the soecific precipitated radioactivity was more than 50 per cent higher and less than double the nonspecific), whereas one of the cultures did not show any synthesis of beta-trace protein (Olsson and Sandberg, unpublished). The culture that did not show any synthesis of beta-trace protein was from a normal person; the culture that showed a probable synthesis was from a patient with multiple sclerosis.

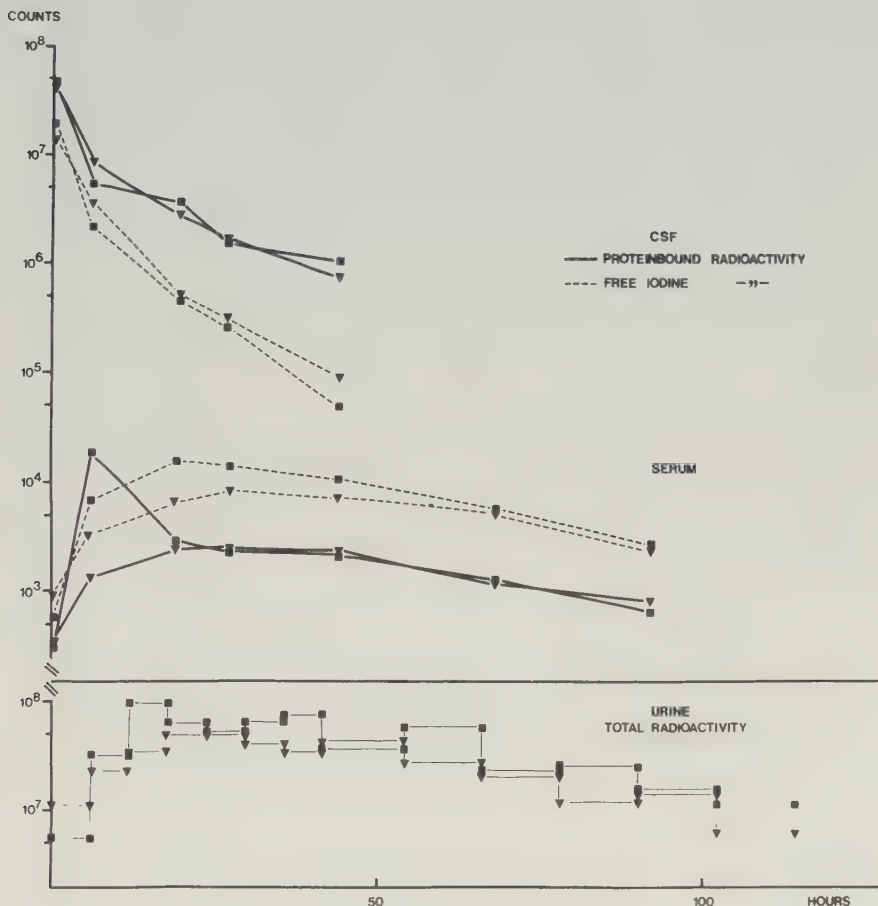


Fig. 6. Curves from the study after injection of ^{125}I -labelled beta-trace protein into the lumbar spinal space in patients No. 3 ($\blacktriangledown - \blacktriangledown$) and No. 4 ($\blacksquare - \blacksquare$). The upper and the middle part of the figure contain curves of the protein-bound and free iodine radioactivity in CSF and serum (counts per 3 ml and 10 min.), respectively, whereas the lower part of the figure contains the urinary excretion of radioactivity (counts per 6 hours).

Discussion:

The metabolic rate of proteins with low molecular weight has been studied in a few other cases, e.g., the Bence Jones proteins with a molecular weight of about 25,000 and the retinol binding protein (RBP) with a molecular weight of 21,000. These proteins have a rapid metabolism; about 20-40 per cent of the Bence Jones proteins are metabolized per hour (Solomon, Waldmann, Fahey and McFarlane, 1964; Jensen, 1970) and the plasma pool of the free RBP has a turnover of approximately 20 times per 24 hours (Vahlquist, 1972). These results agree with the rapid turnover time found intravascularly for beta-trace protein.

The present findings of beta-trace protein in cultures of white CNS matter, basal ganglia, and spinal cord agree with the distribution studies, where significantly larger amounts of beta-trace protein were found in white CNS matter and basal ganglia than in grey CNS matter (paper III). With a somewhat different technique, Hochwald et al. (1967) investigated 12 cultures of brains of rhesus monkeys and found no signs of synthesis of beta-trace protein in 11 of them, whereas one showed a doubtful synthesis. However, the brain material was not dissected into different CNS parts, and perhaps the tissues contained mainly grey matter.

The findings of an extra-CNS synthesis of beta-trace protein predominantly in the genital organs agree with the large amounts of beta-trace protein found in genital organs, especially epididymis (paper II) and the large absolute amounts of beta-trace protein in seminal fluid. Some evidence of synthesis of beta-trace protein in genital organs was found by Hochwald et al. (1967). It is notable that neither eight cultures of ovary in that investigation nor two cultures of ovary in the present investigation showed any sign of synthesis of beta-trace protein.

The synthesis of beta-trace protein in cultures of lymphocytes and the earlier described presence of beta-trace protein in leucocytes (Penny and Osserman, 1971) are difficult to explain, owing to the low concentration of beta-trace protein in serum. Perhaps these investigations indicate some connexion between beta-trace protein and the immunoglobulins, which are probably largely synthesized by the lymphocytes. Other findings that support this hypothesis are the electrophoretic mobility of beta-trace protein in the gamma-globulin region and the molecular weight of beta-trace protein, which is in the neighbourhood of the light chains of the immunoglobulins. Beta-trace protein also contains carbohydrates, about 7-8% glucosamine, 0.9% sialic acid (N-Acetyl Neuraminic Acid), and about 1-2% mannose and galactose (Carlstedt and Olsson, unpublished), which can be compared to the composition of the carbohydrates in human immunoglobulin G, which contains about 2% glucosamine, 1.3% sialic acid, 4.2% mannose and 1% galactose (Acton, Niedermeier, Weinheimer, Clem, Leslie and Bennet, 1972).

BETA-TRACE PROTEIN IN RELATION TO NEUROLOGICAL DISEASES

a. Various neurological disorders:

CSF was obtained from 146 patients with various neurological diseases, such as multiple sclerosis (28), epilepsy (22), paralysis agitans (21), cerebrovascular lesion (20), polyneuropathy (17), cerebral concussion (10), chronic myelopathy (9), intracranial tumour (7), dementia (7), amyotrophic lateral sclerosis (5). The concentration of beta-trace protein in CSF from these patients was compared with healthy, age-matched controls. No significant differences could be found (paper I).

b. Cerebral vascular lesions:

As significantly increased concentrations of beta-trace protein in CSF and urine from patients with cerebrovascular disorders have been reported (Ericsson et al., 1969), a further 28 patients with those diseases were studied (paper I). The pa-

tients were admitted to the Neurological Department after a stroke and were examined with two or more lumbar punctures to get a temporal profile of the concentration of beta-trace protein in CSF during the time after a stroke. The values of beta-trace protein in CSF were found to be fairly constant for the first three weeks; they then increased to reach maximum values during the 5th week after the onset of the stroke. Thereafter the concentration of beta-trace protein in CSF again decreased (Fig. 7).

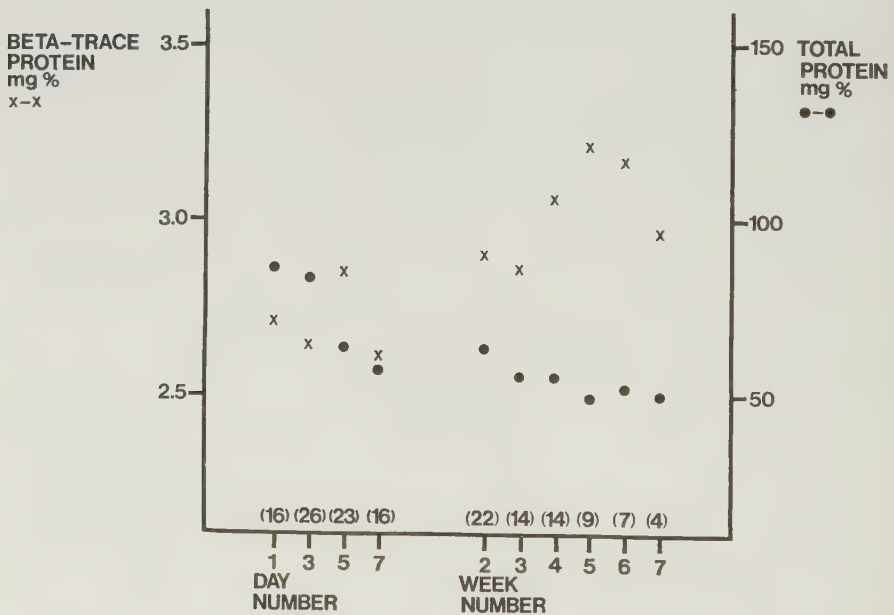


Fig. 7. The mean concentrations of beta-trace protein and total CSF protein at different time intervals after the onset of a stroke in 28 patients. Numbers within parentheses give the number of CSF specimens obtained at the time noted.

There was a significant difference ($0.01 < p < 0.05$) between the values during the 5th week and the 3rd day after the onset of

the stroke. The temporal profile was accentuated when the patients with clinical signs and symptoms of a severe stroke were considered separately. During the study, the total CSF protein concentration decreased (Fig. 7), indicating that the initial blood-CSF barrier was successively repaired. Despite this, the concentration of beta-trace protein in CSF increased, which indicates that the protein was derived from the CNS.

c. Multiple sclerosis:

Slightly increased concentrations of beta-trace protein in CSF from patients with multiple sclerosis have been reported (Link, 1967), whereas the patients investigated in paper I had slightly lower concentrations in CSF than the controls. However, neither of the two groups differed significantly from the controls. When the 28 patients investigated in paper I were divided with respect to the stage of the disease and the degree of disability, slight but not significantly increased values were found in CSF from patients who were considerably disabled or had had an exacerbation within one month of the lumbar puncture. As multiple sclerosis is a disease where the white CNS matter is damaged, variations of the concentration of beta-trace protein in CSF could be expected during different stages of the disease. In order to study this, further 22 patients with multiple sclerosis were investigated with two or more lumbar punctures, both during exacerbations and remissions. Thus, every patient was his own control, and the value during exacerbation could be compared with the value during remission. The mean concentration of beta-trace protein in CSF during exacerbations was 2.9 ± 0.18 and during remissions 2.7 ± 0.13 , whereas the concentrations in serum were 0.4 ± 0.02 during both exacerbation and remissions. The values are given as mean $\pm$ S.E.M. The differences in CSF were not significant (Olsson and Link, 1973).

In order to study the influence of disability on the concentrations of beta-trace protein in CSF and serum, 70 different disabled patients with multiple sclerosis were investigated: 35 of them had no or only slight symptoms of the disease, i.e.

they depended to a large extent on assistance, and their professional and social life was considerably affected. In the two groups, the mean age and the mean duration of the disease were the same (± 4 years). None of them showed any signs of renal failure. The concentration of beta-trace protein in CSF from those with severe disability was higher, 2.9 ± 0.126 (mean $\pm$ S.E.M. than that in CSF from those with no or only slight disability, 2.7 ± 0.154 , but the values did not differ significantly (Olsson, Link and Müller, unpublished). The patients were divided into four groups with respect to the disability and the duration of the disease. Table 1 shows these results.

Table 1. The concentration of beta-trace protein in CSF and serum in patients with multiple sclerosis, with respect to disability and duration of the disease.

Disability		Duration			
		< 10 years (n=33)		> 10 years (n=37)	
No-slight (n=35)	CSF	2.8	± 0.150	2.6	± 0.257
	serum	0.4	± 0.043	0.3	± 0.041
Severe (n=35)	CSF	2.7	± 0.148	3.1	± 0.199
	serum	0.4	± 0.044	0.3	± 0.043

Values are expressed as mg per 100 ml and given as mean $\pm$ S.E.M.

The highest concentrations of beta-trace protein in CSF were found in the group severely disabled by the disease. These values differed significantly ($0.01 < p < 0.05$) from those in the most benign group, i.e. those with no symptoms and a long duration of the disease. The concentrations of beta-trace protein in serum did not differ significantly between the groups of patients.

d. CNS tumours:

As a significantly increased excretion of beta-trace protein in urine from patients with cerebral tumours has been reported (Ericsson et al., 1969), water-soluble extracts of different brain tumours were analysed for the amount of beta-trace protein. Considerably larger amounts of beta-trace protein were found in tumours of glial cell origin, i.e. astrocytomas, oligodendrogliomas, and other gliomas. Beta-trace protein was usually absent or present in unmeasurable amounts in meningiomas, ependymomas, and brain metastatic tumours, even though some of these tumours contained very small amounts of the protein (paper IV). The relation between the concentration of beta-trace protein in gliomas and other brain tumours was 58:1. These findings were not reflected in CSF and serum obtained from 66 patients with brain and spinal tumours. The highest concentrations of beta-trace protein in CSF were found in patients with malignant gliomas, but these values did not differ significantly from the values in patients with other brain tumours or from the values in age-matched controls (paper IV). However, significantly ($0.01 < p < 0.05$) higher concentrations of beta-trace protein were found in CSF from patients with various spinal tumours. These patients also showed a significantly ($0.001 < p < 0.01$) larger amount of the total protein concentration in CSF than the patients with brain tumours.

Discussion:

The attempts to find significant changes in the concentration of beta-trace protein in CSF during different neurological diseases in order to get clinical use of determination of the protein in CSF have so far yielded sparse results. There is probably an increase in the concentration of beta-trace protein in CSF even after small cerebral damages in patients with multiple sclerosis during exacerbations, but the lack of significantly increased concentrations of beta-trace protein probably depends on the rapid turnover rate of the protein in CSF. Possibly, the slight increase in beta-trace protein in CSF during exacerbations of

multiple sclerosis depends on the destruction of white CNS matter, whereas the significantly increased concentration of beta-trace protein in CSF from severely disabled patients with multiple sclerosis might depend on both destruction and repair of white CNS matter. The increased concentrations of beta-trace protein in CSF after a stroke might also be caused by an increased synthesis of beta-trace protein within the CNS during the repair of the damaged brain, as the increase in the protein does not come until five weeks after the onset of the stroke.

The finding of high amounts of beta-trace protein in brain tumours of glial cell origin is similar to the distribution of the S-100 protein in brain tumours (Haglid et al., 1973). Even if beta-trace protein leaks out more into the CSF than S-100 protein, the different content of beta-trace protein in brain tumours could not be demonstrated in CSF as significant differences. This is probably due to the rapid turnover rate of beta trace protein in CSF. The increase of beta-trace protein in lumbar CSF from patients with spinal tumours and a complete or incomplete subarachnoidal blockage, might depend on an increased turnover time of beta-trace protein in the lumbar CSF below the blockage. However, also the total protein concentration in lumbar CSF increases in patients with spinal tumours. Most of this increased protein content consists of transudation of serum proteins from the dilated vascular net below and at the site of the blockage (Lups and Haan, 1954). As the concentration of beta-trace protein in serum is considerably lower than in CSF, it is highly improbable that the increase of beta-trace protein in lumbar CSF from patients with spinal tumours is derived from serum.

However, even if analysis of beta-trace protein in CSF and serum do not provide any information of the type of brain tumour, perhaps chemical analysis of biopsies from brain tumours can complement the histological examination.

GENERAL SUMMARY

In the present thesis, the low molecular weight beta-trace protein, first found and isolated from human CSF, was found to be distributed mainly to the CNS and the genital organs. An immunological identity was also demonstrated between the beta-trace protein in these different tissues, as well as between human and monkey beta-trace protein. In CNS, the protein is predominantly localized to the white matter, being mainly found in cells, such as astrocytes and oligodendrocytes, and in fractions enriched in glial cells, although it also exists to some extent in fractions enriched in neuronal cells. The protein leaks from the CNS to the CSF, the concentration in CSF being about seven times as high as in serum. About 5 per cent of the daily metabolized protein is excreted in urine as unchanged beta-trace protein, whereas the major part of the intravascularly metabolized protein is probably either reabsorbed in the kidneys or excreted as split products. In genital organs, the largest amounts of beta-trace protein are found in extracts of epididymis where the protein is localized to the stroma. The absolute concentration of beta-trace protein in seminal fluid is rather large, whereas the relative concentration is only about one per mille of the total protein content in the fluid.

Beta-trace protein in serum has a short turnover time of about 1.2 hours and about 240 mg is metabolized per 24 hours. Almost all of the protein is excreted in urine. Also the turnover time in CSF is short, being about three hours. The short turnover times in CSF and serum, compared with the seven times as high concentration of beta-trace protein in CSF as in serum, indirectly prove a synthesis of beta-trace protein within the CNS. Synthesis of the protein can also be demonstrated in cultures of white CNS matter from squirrel monkeys and in cultures of human lymphocytes, whereas a probable synthesis was found in cultures of basal ganglia, spinal cord, liver, testis, epididymis, spermatic cord, and fimbriae ovary of the Fallopian tube. With the knowledge of the turnover time and the concentration

of beta-trace protein in the CSF and the CSF volume, it is possible to calculate that about 30 mg beta-trace protein is synthesized within the CNS per 24 hours, i.e. about one eighth of the synthesized human beta-trace protein derives from the CNS.

In pathological conditions, considerably larger amounts of beta-trace protein are found in extracts of water-soluble proteins from brain tumours derived from glial cells than in other brain tumours. Changes of beta-trace protein in CSF and serum from normal values are sparse, probably because of the rapid turnover rates of the protein in these fluids. However, increased concentrations of beta-trace protein in CSF can be seen after strokes and in patients severely disabled with multiple sclerosis probably due to either destruction or repair of the damaged brain tissue.

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